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Selective Inhibition of 11β-Hydroxysteroid Dehydrogenase Type 1 Improves Hepatic Insulin Sensitivity in Hyperglycemic Mice Strains

PĒTERIS ALBERTS, CECILIA NILSSON, GÖRAN SELÉN, LARS O. M. ENGBLOM, NAIMIE H. M. EDLING, SOLVEIG NORLING, GUNNEL KLINGSTRÖM, CATARINA LARSSON, MARGARETA FORSGREN, MANDANA ASHKZARI, CATRINE E. NILSSON, MAJ FIEDLER, ELISABET BERGQVIST, BIRGITTA ÖHMAN, EVA BJÖRKSTRAND, AND LARS B. ABRAHMSÉN

Biovitrum SE12, SE-112 76, Stockholm, Sweden

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been proposed as a new target for type 2 diabetes drugs. The aim of the present study was to assess the effects of inhibition of 11β-HSD1 on blood glucose levels, glucose tolerance, and insulin sensitivity in mouse models of type 2 diabetes. BVT.2733 is an isoform-selective inhibitor of mouse 11β-HSD1. Hyperglycemic and hyperinsulinemic *ob/ob*, *db/db*, KKA^y, and normal C57BL/6J mice were orally administered BVT.2733 (200 mg/kg·d, twice daily). In hyperglycemic, but not in normal mice, BVT.2733 lowered circulating glucose (to 50–88% of control) and insulin (52–65%) levels. In oral glucose tolerance tests in *ob/ob* and KKA^y mice, glucose concentrations were 65–75% of vehicle values after BVT.2733 treatment, and in KKA^y mice insulin concentrations were decreased (62–74%). Euglycemic, hyperinsulinemic clamps demonstrated decreased endogenous glucose production (21–61%). Analysis of hepatic mRNA in KKA^y mice showed reduced phosphoenolpyruvate carboxykinase mRNA (71%). A slight reduction in food intake was observed in *ob/ob* and KKA^y mice. Cholesterol, triglycerides, and free fatty acid levels were decreased to 81–86% in KKA^y mice after a 4-h fast. The results support previous suggestions that selective 11β-HSD1 inhibitors lower blood glucose levels and improve insulin sensitivity in different mouse models of type 2 diabetes. (*Endocrinology* 144: 4755–4762, 2003)

IT IS WELL established that glucocorticoids oppose the insulin effect in regulation of carbohydrate metabolism *in vivo*, including glucose uptake in peripheral tissues and hepatic glucose production (1). The liver plays a central role in maintaining glucose homeostasis, and patients with type 2 diabetes have increased hepatic glucose production, mainly due to increased gluconeogenesis (2). Glucocorticoids upregulate the genes encoding phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme for gluconeogenesis, and glucose-6-phosphatase (G6Pase), which regulates the outflow of glucose originating from either gluconeogenesis or glycogen degradation (3, 4). In man, iv cortisol infusion increases postabsorptive plasma glucose, insulin, and gluconeogenesis (5). The detrimental effects of chronic exposure to high circulating glucocorticoid levels are clearly illustrated by excess hepatic glucose production and type 2 diabetes observed in Cushing's syndrome (1).

Glucocorticoids exert their tissue effects by binding to the glucocorticoid and mineralocorticoid receptors, both members of the nuclear receptor superfamily of ligand-activated transcription factors. Administration of the glucocorticoid receptor antagonist RU 486 decreases blood glucose in *db/db* mice and in patients with Cushing's syndrome (6, 7). However, the specific effects on target tissues depend not only on receptor type and density, but also on the availability and metabolic conversion of glucocorticoid by intracellular enzymes (8). Two 11β-hydroxysteroid dehydrogenases (11β-HSD) catalyze the interconversion between active and inactive glucocorticoids (9). The enzyme 11β-HSD1 is widely expressed and yields increased local tissue concentration of active glucocorticoid by converting cortisone into cortisol in humans, and 11-dehydrocorticosterone into corticosterone in rodents. In contrast, the enzyme 11β-HSD2 catalyzes the opposite reaction, the inactivation of active glucocorticoid (10, 11).

The phenotype of mice having a targeted disruption of the 11β-HSD1 gene substantiates the role of 11β-HSD1 in the liver where it is highly expressed (12, 13). These mice have slightly lower plasma glucose levels on high-fat diet and show attenuated activation of key enzymes for hepatic gluconeogenesis after 24-h starvation combined with stress. Furthermore, 11β-HSD1 knockout mice exhibit improved glucose tolerance, which is displayed despite modestly elevated plasma corticosterone levels. This shows the pivotal role of 11β-HSD1 for enhancing intracellular glucocorticoid action *in vivo*.

BVT.2733 is a selective inhibitor of murine 11β-HSD1 shown to decrease blood glucose levels and gluconeogenic enzymes after 7-d continuous sc administration to hyperglycemic KKA^y mice (14). In the present study the effect of oral administration twice a day (b.i.d.) on glucose and insulin

Abbreviations: AUC, Area under the curve; b.i.d., *bis in diem*, twice a day; BVT.2733, 3-chloro-2-methyl-*N*-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl}benzenesulfonamide; EGP, endogenous glucose production; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDR, glucose disposal rate; GIR, glucose infusion rate; G6Pase, glucose-6-phosphatase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; OGTT, oral glucose tolerance test; PEPCK, phosphoenolpyruvate carboxykinase, p.o., *per os*, orally.

levels was tested in both KKA^y mice and two other established animal models of type 2 diabetes, the *ob/ob* and the *db/db* mice. Furthermore, the effect of BVT.2733 on endogenous glucose production, and insulin sensitivity was assessed in euglycemic, hyperinsulinemic clamp studies and glucose tolerance tests in conscious *ob/ob* and KKA^y mice.

Materials and Methods

Chemical substance

The compound 3-chloro-2-methyl-*N*-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl} benzenesulfonamide (BVT.2733) (15) was dissolved in 12% β-hydroxypropylcyclodextrin (Fluka, Buchs, Switzerland) and 0.3% sodium chloride (p.a. grade, Merck & Co., Darmstadt, Germany). Both the compound and vehicle were administered by oral gavage (p.o.) twice daily (b.i.d.) with 12-h intervals at 0700–0800 and 1900–2000 h for 3–4 d.

Animals

Male mice, C57BL/6J, C57BL/6JBom-*ob/ob* (*Lep^{ob}*; *ob/ob*; age 17–20 wk), and C57BL/KSBom-*db/db* (*Lepr^{db}*; *db/db*; age 12–17 wk), from Taconic Farms (Ry, Denmark) were given a normal diet (R34, Lactamin, Vadstena, Sweden) and water *ad libitum*. Male KK-A^y/Ta Jcl (KKA^y) mice (age, 8–14 wk; 10–12 wk old at the time of clamp) from Clea Japan (Tokyo, Japan) were given a high-fat diet (purified version of Sweetened Condensed Milk Diet, catalog no. D12266B; 32 kcal% fat, ~0.01% cholesterol; Research Diets, New Brunswick, NJ) and water *ad libitum*. The animals were kept one per cage at 22 ± 1 C, 50 ± 20% humidity, and a 12-h light, 12-h dark cycle with lights on at 0500–0630 h. They were grouped based on 4-h fasting blood glucose values, 4-h fasting blood glucose and plasma insulin (clamp studies), or body weight (normal, wild-type C57BL/6J mice). The procedures involving animals were in conformity with national and international laws for the care and use of laboratory animals and were approved by the local animal ethical committee.

Serum and plasma preparation

Serum was prepared from trunk blood samples that were kept 30 min at 4 C before centrifugation at 2000–3000 × *g* for 10–15 min and stored in tubes at −70 C until analysis.

Plasma was prepared from tail blood samples (10–20 μl) that were collected in heparinized hematocrit capillary tubes (Kebo-Lab, VWR, Solna, Sweden) on ice, centrifuged (24,000 × *g*, 10 min, 4 C), transferred to a microtiter 96-well plate, and stored at −20 C until analysis.

Glucose analysis

Blood glucose concentrations (Figs. 1–3 and Tables 2 and 3) were measured immediately using microcuvettes and test strips (Hemocue, Ängelholm, Sweden; or Accu-Chek, Roche, Basel, Switzerland). Both methods are based on glucose dehydrogenase conversion of glucose to gluconolactone. The measurable range is 0–22.2 mM for Hemocue and 0.6–33.3 mM for Accu-Chek. The reported within-run precision is SD ≤ 0.3 mM with coefficients of variation of 3.5%, 2.6%, 1.9%, 1.6%, and 2.2% at 4.3, 7.7, 12.5, 18.2, and 21.0 mM glucose, respectively (Hemocue), and with a coefficient of variation less than 4.0% at 9.2 mM and less than 2.9 SD at 3.3 mM glucose (Accu-Chek). When glucose concentrations exceeded the measurable range, samples were diluted with heparin (LEO, Lövens, Ballerup, Denmark; final concentration, 10 IU/ml in physiological saline) or physiological saline.

Serum glucose concentrations (Fig. 1) were measured with an UV method (Roche) using a Cobas Mira instrument. Glucose was oxidized to gluconolactone by glucose dehydrogenase in the presence of NAD^+ and mutarotase that accelerates the reaction. The formation of NADH was measured photometrically at 340 nm.

Insulin, cholesterol, triglyceride, and free fatty acid analysis

Serum insulin was analyzed with a rat insulin RIA (Linco Research, Inc., St. Louis, MO). In the oral glucose tolerance test (OGTT) and clamp experiments, plasma insulin was analyzed with a rat insulin ELISA (Mercodia, Uppsala, Sweden).

Serum cholesterol was analyzed with an enzymatic colorimetric method (MPR2, Roche). Serum triglycerides were analyzed with an enzymatic colorimetric method after elimination of free glycerol (Triglycerides/GB, Roche). Serum nonesterified, free fatty acids were determined with an enzymatic colorimetric method using oleic acid as a standard (NEFA C, Wako Chemicals, Neuss, Germany).

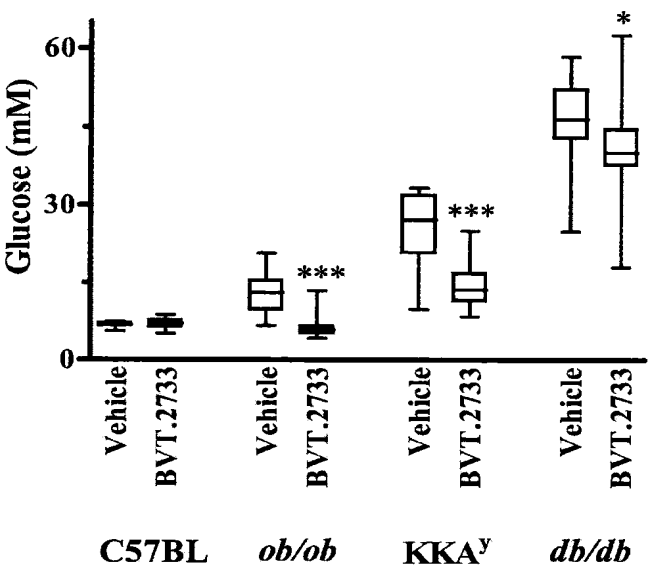

FIG. 1. Effect of BVT.2733 (200 mg/kg·d, p.o.) administration on blood glucose levels in normal C57BL/6J (n = 25 and 25) and spontaneously hyperglycemic *ob/ob* (n = 24 and 25), KKA^y (n = 16 and 16), and *db/db* (n = 22 and 22; serum glucose) mice measured 12 h after the last administration, which was once every 12 h for 4 consecutive days. Statistical difference from control: *, $P < 0.05$; ***, $P < 0.001$.

mRNA analysis

Real-time PCR was used to quantify mRNA levels (TaqMan, PE Applied Biosystems, Foster City, CA) of 11β-HSD1 (EC 1.1.1.146), PEPCK (EC 4.1.1.32), and G6Pase (EC 3.1.3.9) isolated from liver, mesenteric fat, and epididymal fat (14). Levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and 18S rRNA. Plasmid containing cloned reference DNA was used to construct standard curves for 11β-HSD1and GAPDH levels (PE Applied Biosystems).

OGTT

All animals were administered BVT.2733 or vehicle b.i.d.; *ob/ob* mice BVT.2733 (200 mg/kg·d; for 4 d) and KKA^y mice BVT.2733 (400 mg/kg·d; for 3 d). D-Glucose (2 g/kg; 200 mg/ml stock solution, Fresenius Kabi, Uppsala, Sweden) was given p.o. after overnight fasting and 2 h after the last administration of vehicle or BVT.2733. Blood samples were taken immediately before the glucose load and after 15, 30, 60, and 120 min.

Euglycemic, hyperinsulinemic clamp

Surgery and drug treatment before clamp. A microrenathane implantation tubing catheter (Braintree Scientific, Inc., Braintree, MA) was inserted into the right jugular vein under isoflurane (Forene, Abbott, Chicago, IL) anesthesia at least 4 d before administration of drug or vehicle and at least 8 d before the clamp. The catheter was tunneled under the skin, extracted in the neck, and filled with glycerol containing heparin to prevent blood from clotting. BVT.2733 (200 mg/kg·d) or vehicle was administered for 3 d (KKA^y mice) and 4 d (*ob/ob* mice).

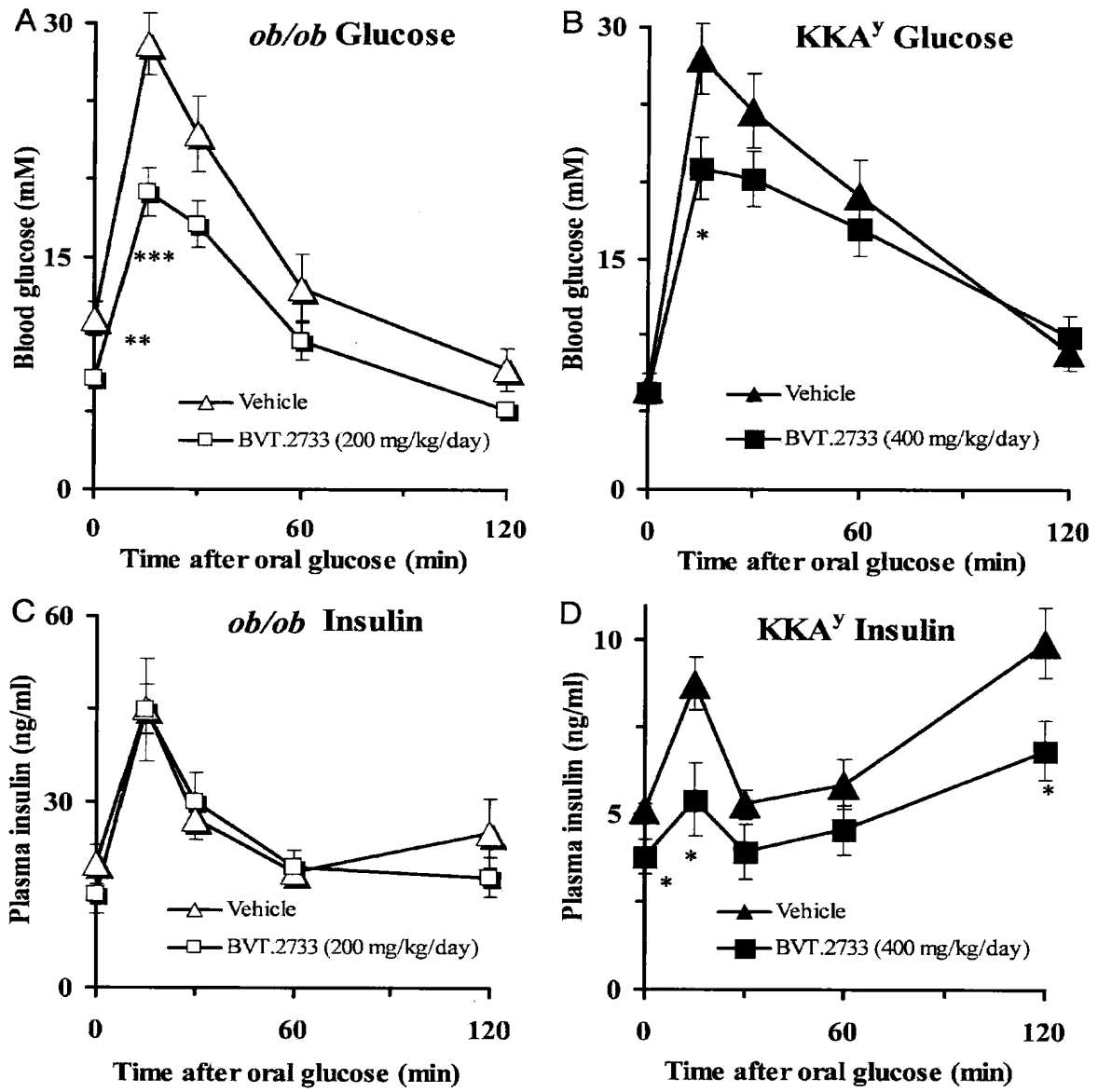

FIG. 2. OGTT. Spontaneously hyperglycemic *ob/ob* (A and C) and KKA^y (B and D) mice were treated with BVT.2733 (n = 12) or vehicle (n = 12), and after an overnight fast (14 h), D-glucose (2 g/kg, p.o.) was administered. A and B, Blood glucose concentrations. C and D, Plasma insulin concentrations. △ and ▲, Vehicle; ■ and □, BVT.2733; △ and □, *ob/ob* mice; ▲ and ■, KKA^y mice. Statistical difference from control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Clamp protocol. The clamps were performed in conscious mice (16). After administration of the last BVT.2733 dose the animals were fasted for 5 h before start of the clamp procedure. The animals were connected to a liquid swivel via the venous catheter and acclimatized for 45 min.

3-D-[^{3}H]Glucose tracer (PerkinElmer, Boston, MA) dissolved in 0.9% saline was infused throughout the clamp to determine the glucose disposal rate (GDR). A priming bolus dose of 25 μCi/kg (925 kBq/kg) was given, followed by a continuous infusion of 2.5 μCi/kg·min (infusion rate, 50 μl/min·kg) by a microinjection pump (CMA/Microdialysis, Solna, Sweden). Blood samples (10 μl) were collected after 50, 60, and 70 min of D-[^{3}H]glucose infusion and were used to determine the basal GDR. At the same time points, basal blood glucose levels were measured, and at 70 min a 20-μl blood sample was taken for basal plasma insulin measurement.

Insulin infusion (Actrapid, Novo Nordisk, Bagsværd, Denmark) was started 70 min after initiation of D-[^{3}H]glucose infusion and continued for 90 min at a constant rate. A relatively low insulin concentration (12.5 mU/kg·min) was used to prevent complete suppression of endogenous glucose production (EGP) and therefore optimized to evaluate hepatic insulin sensitivity. Blood samples (10 μl) were taken after 70, 80, and 90 min of insulin infusion for determination of GDR. At 90 min, a 20-μl blood sample was taken for determination of circulating insulin levels during insulin infusion.

D-Glucose (300 mg/ml; Fresenius Kabi) was administered by an ad-

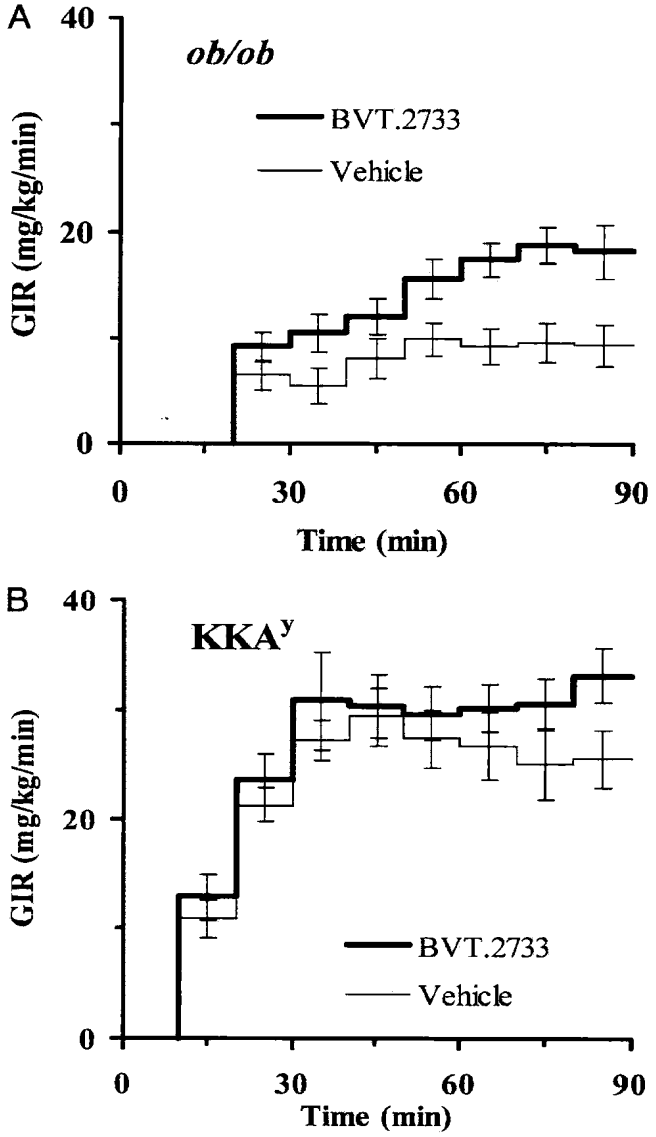

FIG. 3. GIR during euglycemic, hyperinsulinemic clamp in *ob/ob* mice (A; n = 10) and KKA^y mice (B; n = 8) after treatment with BVT.2733 (200 mg/kg·d, p.o.; *thick line*) or vehicle (*thin line*). For statistical differences at steady-state insulin infusion conditions (at 70–90 min), see Table 3.

justable infusion pump (model 100, KD Scientific, New Hope, PA) to maintain blood glucose at the same level as before insulin infusion. The glucose infusion rate (GIR) was guided by blood glucose concentration measurements every 10 min. Blood glucose was clamped at the individual basal blood glucose level.

Blood [^{3}H]D-glucose. Barium hydroxide (50 μl, 0.3 M) and zinc sulfate (50 μl, 0.3 M) were added to blood samples (10 μl) and mixed. After centrifugation, supernatant (25-μl) samples in duplicate were transferred to scintillation vials and allowed to evaporate to dryness overnight to eliminate [^{3}H]water. Next day, D-[^{3}H]glucose was dissolved in water (0.5 ml), scintillation fluid (3 ml Ultima Gold, Packard, Meriden, CT) was added, and radioactivity was measured in a liquid scintillation spectrometer (TriCarb, Packard, Meriden, CT).

Calculations. Blood glucose specific activity was calculated as the D-[^{3}H]glucose divided by the measured blood glucose levels. Steady-state conditions for blood glucose specific activity were achieved during basal and clamp periods in both *ob/ob* mice and KKA^y mice, as determined by linear regression analysis. The slopes were not significantly different from zero for all groups ($P > 0.05$; data not shown). GDR was calculated by dividing the rate of D-[^{3}H]glucose infusion by the average specific activity of blood glucose. During basal conditions, EGP is assumed to be equal to the GDR. Under steady-state insulin infusion conditions (at 70–90 min), it is assumed that EGP = GDR − GIR.

Results are expressed as the mean ± SEM, except in Figs. 1 and 4, where the box extends from the 25th to the 75th percentile, with a line at the median, and the error bars show the highest and lowest values (PRISM 3.0 software, GraphPad, San Diego, CA).

Statistics

Data from the OGTT and clamp were analyzed in two ways. OGTT area under the curve (AUC) data were calculated using a trapezoidal method with baseline adjustments. Clamp GIR AUC data were calculated using a rectangular method. Differences in AUC between compound- and vehicle-treated animals were analyzed using two-tailed, two-sample, equal variance (independent samples) *t* tests. Further, data were analyzed using repeated measurements ANOVA. In regression analyses, concerning analyses of calculated clamp EGP and GDR, the insulin-stimulated values were used as dependent variables, and basal values and treatment (0/1) were used as covariates. The nonparametric Mann-Whitney test was used when data significantly deviated from a normal distribution according to a Kolmogorov-Smirnov test. The glucose data were statistically assessed by independent sample *t* test. The mRNA data were statistically assessed by nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test.

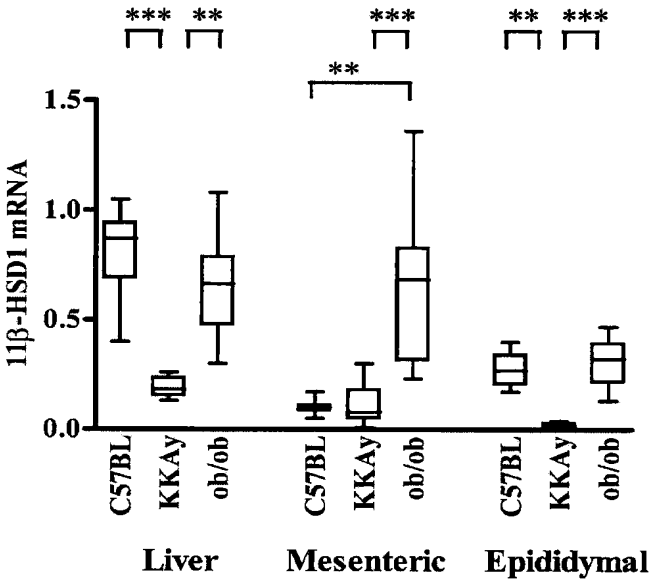

FIG. 4. Levels of 11β-HSD1 mRNA in C57BL/6J, KKA^y, and *ob/ob* mice given as relative units *vs.* GAPDH mRNA in liver, mesenteric fat, and epididymal fat (n = 9–10). Statistical difference is: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Results

Effect of the 11β-HSD1 inhibitor BVT.2733 on glucose, insulin, cholesterol, triglyceride, and free fatty acid concentrations; food intake; and body weight

BVT.2733 (200 mg/kg·d) administered by oral gavage (p.o.) b.i.d. for 4 d reduced circulating glucose concentrations in spontaneously hyperglycemic *ob/ob*, KKA^y, and *db/db* mice to 50%, 58%, and 88% of that vehicle-treated mice (Fig. 1). In contrast, BVT.2733 (200 mg/kg·d) did not alter the blood glucose concentration in normal C57BL/6J mice (Fig. 1).

Serum insulin concentrations were lowered by the BVT.2733 treatment in *ob/ob* and *db/db* mice and tended to be lowered in KKA^y mice, but were not altered in C57BL/6J mice (Table 1A).

Oral BVT.2733 (200 mg/kg·d) administration did not consistently alter serum cholesterol, triglyceride, and free fatty acid concentrations under *ad libitum*-fed conditions. However, cholesterol, triglycerides and free fatty acid levels were decreased in the KKA^y mouse after a 4-h fast (Table 1B). The fasting blood glucose was 69% of the control value.

Daily food intake was not affected in C57BL/6J and *db/db* mice by BVT.2733 (200 mg/kg·d). In *ob/ob* and KKA^y mice, food intake was decreased by 25–39% and 14–19% compared with vehicle. Body weight was not altered in C57BL/6J, KKA^y, and *db/db* mice, but was reduced by 5% in *ob/ob* mice compared with vehicle.

Effect of the 11β-HSD1 inhibitor BVT.2733 on hepatic mRNA concentrations

Treatment of KKA^y mice with BVT.2733 resulted in a dose-dependent reduction of PEPCK mRNA levels in the liver from 2.12 ± 0.16 for vehicle to 1.95 ± 0.10 and 1.51 ± 0.14 ($P < 0.01$; n = 8) after the administration of 100 and 200 mg/kg·d, respectively (relative units from normalization to GAPDH mRNA). The levels of 11β-HSD1 mRNA and G6Pase mRNA also tended to decrease. The level of 11β-HSD1 mRNA was 2.45 ± 0.44 for vehicle and 2.33 ± 0.25 and 1.75 ± 0.21 for BVT.2733 (100 and 200 mg/kg·d; n = 8). The level of G6Pase mRNA was 1.09 ± 0.19 for vehicle and 1.55 ± 0.19 and 0.78 ± 0.13 for BVT.2733 (100 and 200 mg/kg·d; n = 8). Similar results were obtained when data were normalized to 18S rRNA (not shown).

Effect of the 11β-HSD1 inhibitor BVT.2733 on OGTT

OGTTs were performed in *ob/ob* and KKA^y mice. In mice from both strains BVT.2733 treatment resulted in decreased blood glucose concentrations at early time points compared with vehicle treatment (Fig. 2). In the KKA^y mouse this was accompanied by lower insulin concentrations both before and after glucose administration, but in the *ob/ob* mice no differences in the effect on insulin levels were seen. ANOVAs using repeated measurements did not yield any significant differences between drug-treated and vehicle-treated mice. Also, the baseline adjusted AUCs (data not shown) for glucose and insulin in the KKA^y and *ob/ob* BVT.2733-treated and vehicle-treated mice did not differ significantly (independent sample t tests). In the KKA^y mice BVT.2733 (400 mg/kg·d) was used, because at 200 mg/kg·d no effect on the OGTT was observed in a pilot experiment (data not shown).

Effect of the 11β-HSD1 inhibitor BVT.2733 on insulin sensitivity

Blood glucose concentrations during steady-state insulin infusion in the euglycemic, hyperinsulinemic clamp were similar to basal levels in *ob/ob* mice and vehicle-treated KKA^y mice, but lower than basal levels in BVT.2733-treated KKA^y mice (Table 2). Plasma insulin was increased approximately 2- to 4-fold by the insulin (12.5 mU/kg·min) infusion (Table 2).

The GIR in BVT.2733-treated mice was higher than that in vehicle-treated mice at all time points in both *ob/ob* and KKA^y mice (Fig. 3 and Table 3). In *ob/ob* mice the corresponding AUCs between BVT.2733- and vehicle-treated mice were also significantly different ($P < 0.01$; data not shown).

TABLE 1. Effect of BVT.2733 administration on serum insulin, cholesterol, triglyceride, and free fatty acid concentrations

Mouse strain	Insulin (ng/ml)	Cholesterol (mM)	Triglycerides (mM)	Free fatty acids (mM)	n
A. Effect of 4-d BVT.2733 (200 mg/kg·d) administration on *ad libitum*-fed serum concentrations 12 h after dose					
C57BL/6J					
Vehicle	1.0 ± 0.078	2.9 ± 0.064	1.1 ± 0.043	0.65 ± 0.032	24
BVT.2733	1.2 ± 0.15	2.9 ± 0.081	1.0 ± 0.045	0.63 ± 0.035	25
ob/ob					
Vehicle	96.3 ± 12.3	6.0 ± 0.15	1.1 ± 0.082	0.67 ± 0.031	24
BVT.2733	59.7 ± 11.6[a]	6.5 ± 0.17[a]	0.91 ± 0.038	0.47 ± 0.025[b]	25
KKA^y					
Vehicle	32.8 ± 5.2	4.1 ± 0.21	3.1 ± 0.32	1.5 ± 0.10	15
BVT.2733	21.4 ± 2.4	3.3 ± 0.14	2.2 ± 0.15	1.1 ± 0.058	16
db/db					
Vehicle	2.7 ± 0.50	2.6 ± 0.13	1.2 ± 0.10	0.81 ± 0.046	22
BVT.2733	1.4 ± 0.32[a]	2.7 ± 0.15	1.2 ± 0.12	1.1 ± 0.083[a]	22
B. Effect of 3-d BVT.2733 (200 mg/kg·d) administration on 4-h fasting serum concentrations 4 h after dose					
KKA^y					
Vehicle	11.8 ± 1.1	4.2 ± 0.11	2.4 ± 0.16	0.90 ± 0.042	30
BVT.2733	10.6 ± 0.96	3.6 ± 0.14[b]	2.0 ± 0.10[a]	0.73 ± 0.029[c]	29

The same animals were used as those in Fig. 1.
[a] $P < 0.05$.
[b] $P < 0.001$.
[c] $P < 0.01$.

TABLE 2. Blood glucose and plasma insulin concentrations at steady state during euglycemic, hyperinsulinemic clamp for vehicle, and BVT.2733 (200 mg/kg·d)

Mouse strain	Blood glucose (mM)		Plasma insulin (ng/ml)	
	Basal	Insulin infusion	Basal	Insulin infusion
ob/ob				
Vehicle (n = 10)	7.3 ± 0.83	7.4 ± 0.79	24.3 ± 5.8	66.1 ± 13.2[a]
BVT.2733 (n = 10)	5.4 ± 0.39	5.4 ± 0.47[b]	20.2 ± 4.2	38.5 ± 10.5[c]
KKA^y				
Vehicle (n = 8)	14.0 ± 1.9	13.7 ± 1.8	8.4 ± 1.8	25.9 ± 1.8[a]
BVT.2733 (n = 8)	10.9 ± 2.1	9.7 ± 1.9[d]	6.7 ± 2.0	25.2 ± 3.0[a]

[a] $P < 0.001$ *vs.* basal.
[b] $P < 0.05$ *vs.* vehicle.
[c] $P < 0.05$ *vs.* basal.
[d] $P < 0.01$ *vs.* basal.

TABLE 3. Effect of BVT.2733 (200 mg/kg·d) on glucose disposal rate (GDR), glucose infusion rate (GIR), and endogenous glucose production (EGP)

Mouse strain	GDR (mg/kg·min)		GIR (mg/kg·min)	EGP (mg/kg·min)	
	Basal	Insulin infusion	Insulin infusion	Basal	Insulin infusion
ob/ob					
Vehicle (n = 10)	27.8 ± 4.9	28.7 ± 1.7	9.4 ± 1.7	27.8 ± 4.9	19.4 ± 2.3
BVT.2733 (n = 10)	25.5 ± 4.0	29.5 ± 1.2	17.7 ± 1.7[a]	25.5 ± 4.0	11.8 ± 1.6[b,c]
KKA^y					
Vehicle (n = 8)	35.1 ± 4.9	44.3 ± 5.2[d]	25.7 ± 2.9	35.1 ± 4.9	18.5 ± 3.6[b]
BVT.2733 (n = 8)	26.1 ± 2.8	35.1 ± 2.4[b]	31.3 ± 2.3	26.1 ± 2.8	3.8 ± 1.0[e]

The GDR, GIR, and EGP values were calculated from samples obtained after 70–90 min of insulin infusion (*cf.* Fig. 3).
[a] $P < 0.01$ *vs.* vehicle.
[b] $P < 0.01$ *vs.* basal.
[c] $P < 0.05$ *vs.* vehicle.
[d] $P < 0.05$ *vs.* basal.
[e] $P < 0.001$ *vs.* basal. $P < 0.001$ *vs.* vehicle.

The GDR was increased during insulin infusion in both vehicle- and BVT.2733-treated KKA^y mice, but was not different between vehicle- and BVT.2733-treated *ob/ob* mice (Table 3). Under basal conditions no difference was observed between vehicle- and BVT.2733-treated groups in any of the strains.

EGP under basal conditions tended to be lower in BVT.2733-treated than in vehicle-treated *ob/ob* and KKA^y mice (Table 3). During insulin infusion, however, EGP was more suppressed in both *ob/ob* and KKA^y mice treated with BVT.2733 compared with vehicle-treated mice (Table 3).

Comparison of 11β-HSD1 mRNA levels in liver and two different fat depots

The two hyperglycemic models in which the effects of 11β-HSD1 inhibition were studied most extensively were further characterized in terms of 11β-HSD1 mRNA levels. The levels of 11β-HSD1 mRNA (normalized to GAPDH mRNA) in the liver, mesenteric fat, and epididymal fat were determined in C57BL/6J, KKA^y, and *ob/ob* mice (Fig. 4). In liver and epididymal fat, the levels were higher in C57BL/6J and *ob/ob* than in KKA^y mice. In mesenteric fat the levels in *ob/ob* mice were higher than those in C57BL/6J and KKA^y mice.

Discussion

Excess tissue glucocorticoid action may underlie several characteristics of type 2 diabetes and the metabolic syndrome. Selective inhibition of 11β-HSD1 provides the means to block local activation of glucocorticoids. The selective 11β-HSD1 inhibitor BVT.2733 has recently been shown to decrease blood glucose, serum insulin, and hepatic PEPCK mRNA levels in hyperglycemic and hyperinsulinemic KKA^y mice when administered continuously by sc osmotic minipumps (14). The present study aimed to investigate the physiological mechanisms behind the effects of 11β-HSD1 inhibition on glucose homeostasis and insulin sensitivity and to explore the effects of oral BVT.2733 administration in three different hyperglycemic and hyperinsulinemic mouse strains, *ob/ob*, *db/db*, and KKA^y, as well as in normal C57BL/6J mice.

The present results show that oral BVT.2733 administration decreased circulating glucose levels in *ob/ob*, *db/db*, and KKA^y mice, with a parallel decrease in insulin levels. Analysis of hepatic mRNA in the KKA^y mouse showed reduced levels of PEPCK mRNA. Thus, the results using oral administration confirm and extend the results obtained after osmotic minipump administration in the KKA^y mouse (14). In the current study a larger effect of 11β-HSD1 inhibition on blood glucose was seen in *ob/ob* and KKA^y mice compared with *db/db* and normal mice. The *ob/ob* mice displayed the same concentration of 11β-HSD1 mRNA in the liver as normal mice, whereas KKA^y mice had only 25%. Furthermore, it has been reported that both the 11β-HSD1 activity and mRNA levels are elevated in livers of *db/db* mice (17). Thus, the magnitude of the reduction of hepatic glucose production and blood glucose levels depends not only on the level of the target enzyme, but on other factors as well. In accordance, no

blood glucose decrease was observed in normal mice despite high levels of 11β-HSD1 expression in the liver. The lack of hypoglycemic effect in normal mice is in agreement with previous data showing that the nonspecific 11β-HSD1 inhibitor carbenoxolone does not yield decreased blood glucose in healthy human volunteers (18, 19). Furthermore, 11β-HSD1 knockout mice display normal fasting glucose levels (12, 20). Thus, inhibition and lack of the enzyme yield similar results. Taken together, the data support a fine-tuning effect of 11β-HSD1 on blood glucose levels, where inhibition leads to a decrease in excessive, but not basal, levels.

After a 4-h fast, blood glucose as well as serum cholesterol, triglycerides, and free fatty acids were decreased in KKA^y mice given BVT.2733. However, samples taken under *ad libitum*-fed conditions did not consistently alter serum cholesterol, triglyceride, or free fatty acid concentrations. As the fasting samples were obtained 4 h after administration, whereas samples from *ad libitum*-fed animals were collected 12 h post treatment, higher levels of inhibitor may have contributed to the decreases in lipids. Thus, lipid lowering might have a beneficial effect on hepatic insulin sensitivity and contribute to the reduced blood glucose levels. A lipid-lowering effect from 11β-HSD1 inhibition was expected based on the lipolysis-inducing properties of glucocorticoids (19, 21). Similarly, selective overexpression of 11β-HSD1 in white adipose tissue leads to increased adipose levels of corticosterone, lipoprotein lipase mRNA, and circulating free fatty acids (22). These transgenic mice develop visceral obesity similar to the human metabolic syndrome, supporting the hypothesis that 11β-HSD1 inhibition might prevent or reduce omental obesity in human metabolic syndrome and type 2 diabetes. Clinical data suggest that overexpression of 11β-HSD1 might contribute to development of the metabolic syndrome, because increased obesity correlates with higher expression of the enzyme (23, 24). However, in humans only sc adipose tissue has been investigated, not the more metabolically active and glucocorticoid receptor-rich visceral adipose tissue, where fat accumulation has been shown to correlate with increased morbidity and mortality (25).

Hyperglycemia and hyperphagia appear to depend on glucocorticoids in both man (23) and the commonly used models for type 2 diabetes *ob/ob*, *db/db*, and KKA^y mice and *fa/fa* Zucker rats (26–28). In *ob/ob* and *db/db* mice, adrenalectomy slows and cortisone treatment increases weight gain (29). Likewise, the glucocorticoid receptor antagonist RU 486 prevents development of hyperphagia, obesity, and fat deposition in *fa/fa* Zucker rats with no effect in lean rats (30, 31). Moreover, mice exhibiting enhanced glucocorticoid activity due to selective overexpression of 11β-HSD1 in white adipose tissue are both hyperglycemic and hyperphagic (22). In accordance, the present study shows that 11β-HSD1 inhibition yielded slightly decreased food intake in *ob/ob* and KKA^y mice. This is expected to contribute to reduced blood glucose levels. However, in KKA^y mice the reduction in food intake was smaller than that in blood glucose, and in *db/db* mice glucose levels were reduced, with no concomitant effect on food intake. Thus, a beneficial reduction in food intake may contribute to the blood glucose-lowering effect of 11β-HSD1 inhibition, although to a different degree in different models of type 2 diabetes.

Glucocorticoids contribute to increased hepatic glucose output in diabetes (4) and counteract the actions of insulin (1). The enzyme 11β-HSD1 enhances the effect by generating active glucocorticoid in target tissues (9). We have previously shown that BVT.2733 inhibits 11β-HSD1 in the liver and reduces hepatic levels of mRNA corresponding to key gluconeogenic enzymes (14). The present results support the hypothesis that selective 11β-HSD1 inhibition yields enhanced insulin sensitivity and agrees with the clinical finding of improved whole body insulin sensitivity using the nonselective 11β-HSD1 inhibitor carbenoxolone (18). The euglycemic, hyperinsulinemic clamp data from the present study show improvement in hepatic insulin sensitivity in both *ob/ob* and KKA^y mice; in both models, EGP was more suppressed during insulin infusion in mice treated with BVT.2733 than in vehicle-treated control animals. Also under basal conditions EGP tended to be lower in BVT.2733-treated *ob/ob* and KKA^y mice. The GIR was higher in *ob/ob* mice treated with the selective 11β-HSD1 inhibitor compared with the vehicle-treated group, and in KKA^y mice treatment with BVT.2733 tended to increase the GIR. In the latter case the GIR may have been underestimated, as the animals did not fully maintain basal glucose levels at steady-state insulin infusion conditions.

The GDR increased during insulin infusion in both vehicle- and BVT.2733-treated KKA^y mice, whereas no effect of BVT.2733 administration was detected. However, a tendency toward increased GDR was seen in *ob/ob* mice after BVT.2733 treatment, which might be a reflection of higher levels of the target 11β-HSD1 in adipose tissue compared with KKA^y mice. Furthermore, preliminary observations of 11β-HSD1 inhibition by BVT.2733 in adipose tissue suggest that peripheral effects are likely (data not shown). However, the primary objective of the present study was to evaluate the effect on hepatic insulin sensitivity. Therefore, mild hyperinsulinemia was employed in the clamp studies to avoid complete suppression of hepatic glucose production. A more pronounced hyperinsulinemia would have been used to reveal effects on peripheral insulin sensitivity. Such levels were employed in a pilot study where insulin was infused at 25 mU/kg·min in KKA^y mice treated with BVT.2733 (400 mg/kg·d) for 3 d. In that study enhanced hepatic insulin sensitivity (EGP) as well as a trend toward increased GDR were seen (data not shown).

In *ob/ob* and KKA^y mice BVT.2733 treatment resulted in reduced glucose levels that were accompanied by reduced or unchanged insulin levels during the OGTT. This suggests improved whole body glucose tolerance and increased insulin sensitivity. The results are in accordance with data on 11β-HSD1 gene knockout mice exhibiting improved glucose tolerance (13) and with impaired glucose tolerance in transgenic mice having increased 11β-HSD1 activity in white adipose tissue (22). A higher dose of BVT.2733 (400 mg/kg·d) was required for effects on glucose tolerance in the KKA^y model, in which a larger effect on insulin and a lesser effect on glucose levels were observed. Thus, there are qualitative differences between the two models, which may reflect different levels of target enzyme not only in liver and adipose tissue, but also in skeletal muscle and pancreas.

In conclusion, oral administration of a selective 11β-HSD1

inhibitor resulted in lowered circulating glucose and insulin levels in three separate mouse models of type 2 diabetes, but not in normal mice, yielding further support to the suggestion that a selective inhibitor of human 11β-HSD1 may lower blood glucose levels in subjects with type 2 diabetes (14) without causing hyperglycemia. Decreased EGP suggests enhanced insulin sensitivity. In addition, a reduction in food intake was seen in some of the models, suggesting a beneficial reduction in weight gain over time.

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Address all correspondence and requests for reprints to: Dr. P. Alberts, Pharmacology 2, Department of Biology, Biovitrum SE12, SE-112 76 Stockholm, Sweden. E-mail: peteris.alberts@biovitrum.com.

Part of the results has been presented in preliminary form (32, 33).

P.A. and C.N. contributed equally to the study.

References

1. **Andrews RC, Walker BR** 1999 Glucocorticoids and insulin resistance: old hormones, new targets. Clin Sci 96:513–523
2. **DeFronzo RA** 1997 Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 5:177–269
3. **Pilkis SJ, Granner DK** 1992 Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 54:885–909
4. **Friedman JE, Yun JS, Patel YM, McGrane MM** 1993 Glucocorticoids regulate the induction of phosphoenolpyruvate carboxykinase. J Biol Chem 268:12952–12957
5. **Rizza RA, Mandarino LJ, Gerich JE** 1982 Cortisol-induced insulin resistance in man: impaired suppression of glucose. J Clin Endocrinol Metab 54:131–138
6. **Nieman LK, Chrousos GP, Kellner C, Spitz IM, Nisula BC, Cutler GB, Merriam GR, Bardin CW, Loriaux DL** 1985 Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486. J Clin Endocrinol Metab 61:536–540
7. **Friedman JE, Sun Y, Ishizuka T, Farrell CJ, McCormack SE, Herron LM, Hakimi P, Lechner P, Yun JS** 1997 Phosphoenolpyruvate carboxykinase (GTP) gene transcription and hyperglycemia are regulated by glucocorticoids in genetically obese *db/db* transgenic mice. J Biol Chem 272:31475–31481
8. **Nobel S, Abrahmsén L, Oppermann U** 2001 Metabolic conversion as a prereceptor control mechanism for lipophilic hormones. Eur J Biochem 268:4113–4125
9. **Seckl JR, Chapman KE** 1997 Medical and physiological aspects of the 11β-hydroxysteroid dehydrogenase. Eur J Biochem 249:361–364
10. **Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER, Monder C** 1988 Localisation of 11β-hydroxysteroid dehydrogenase: tissue specific protector of the mineralocorticoid receptor. Lancet 2:986–989
11. **Funder JW, Pearce PT, Smith R, Smith AI** 1988 Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science 242:583–585
12. **Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CRW, Seckl JR, Mullins JJ** 1997 11β-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. Proc Natl Acad Sci USA 94:14924–14929
13. **Morton NM, Holmes MC, Fiévet C, Staels B, Tailleux A, Mullins JJ, Seckl JR** 2001 Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11β-hydroxysteroid dehydrogenase type 1 null mice. J Biol Chem 276:41293–41300
14. **Alberts P, Engblom L, Edling N, Forsgren M, Klingström G, Larsson C, Rönquist-Nii Y, Öhman B, Abrahmsén L** 2002 Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. Diabetologia 45:1528–1532
15. **Barf T, Vallgårda J, Emond R, Häggström C, Kurz G, Nygren A, Larwood V, Mosialou E, Axelsson K, Olsson R, Engblom L, Edling N, Rönquist-Nii Y, Öhman B, Alberts P, Abrahmsén L** 2002 Arylsulfonamidothiazoles as a new class of potential antidiabetic drugs. Discovery of potent and selective inhibitors of the 11β-hydroxysteroid dehydrogenase type 1. J Med Chem 45:3813–3815
16. **Ren JM, Marshall BA, Mueckler MM, McCaleb M, Amatruda JM, Shulman GI** 1995 Overexpression of Glut4 protein in muscle increases basal and insulin-stimulated whole body glucose disposal in conscious mice. J Clin Invest 95:429–432
17. **Aoki K, Homma M, Hirano T, Oka K, Satoh S, Mukasa K, Ito S, Sekihara H** 2001 mRNA and enzyme activity of hepatic 11β-hydroxysteroid dehydrogenase type 1 are elevated in C57BL/KsJ-*db/db* mice. Life Sci 69:2543–2549
18. **Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards** 1995 Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. J Clin Endocrinol Metab 80:3155–3159.
19. **Andrews RC, Rooyackers O, Walker BR** 2003 Effects of the 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. J Clin Endocrinol Metab 88:285–291
20. **Holmes MC, Kotelevtsev Y, Mullins JJ, Seckl JR** 2001 Phenotypic analysis of mice bearing targeted deletions of 11β-hydroxysteroid dehydrogenases 1 and 2 genes. Mol Cell Endocrinol 171:15–20
21. **Ottosson M, Vikman-Adolfsson K, Enerbäck S, Olivecrona G, Björntorp P** 1994 The effects of cortisol on the regulation of lipoprotein lipase activity in human adipose tissue. J Clin Endocrinol Metab 79:820–825
22. **Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS** 2001 A transgenic model of visceral obesity and the metabolic syndrome. Science 294:2166–2170
23. **Rask E, Olsson T, Söderberg S, Andrew R, Livingstone DE, Johnson O, Walker BR** 2001 Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 86:1418–1421
24. **Rask E, Walker BR, Söderberg S, Livingstone DEW, Eliasson M, Johnson O, Andrew R, Olsson T** 2002 Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11β-hydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab 87:3330–3336
25. **Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, Adams PW** 1982 Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 54:254–260
26. **Bray GA, York DA** 1979 Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev 59:719–809
27. **Livingstone DE, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR** 2000 Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 141:560–563
28. **Walker BR** 2000 Cortisol in insulin resistance syndromes. In: Walker M, Butler P, Rizza RA, eds. The diabetes annual. New York: Elsevier; vol 13:137–157
29. **Shimomura Y, Bray GA, Lee M** 1987 Adrenalectomy and steroid treatment in obese (*ob/ob*) and diabetic (*db/db*) mice. Horm Metab Res 19:295–299
30. **Langley SC, York DA** 1990 Effects of antiglucocorticoid RU 486 on development of obesity in obese *fa/fa* Zucker rats. Am J Physiol 259:R539–R544
31. **Okada S, York DA, Bray GA** 1992 Mifepristone (RU 486), a blocker of type II glucocorticoid and progestin receptors, reverses a dietary form of obesity. Am J Physiol 262:R1106–R1110
32. **Alberts P, Engblom L, Edling N, Forsgren M, Klingström G, Larsson C, Rönquist-Nii Y, Öhman B, Abrahmsén L,** The selective 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitor BVT. 2733 administered orally lowers blood glucose levels in hyperglycemic KKAy, *ob/ob*, and *db/db* mice. Program of the 84th Annual Meeting of The Endocrine Society, San Francisco, CA, 2002, p 583 (Abstract P3-392)
33. **Nilsson C, Abrahmsén L, Selén G, Nilsson CE, Norling S, Alberts P, Björkstrand E,** Increased hepatic insulin sensitivity after treatment with the 11β-hydroxysteroid dehydrogenase 1 inhibitor BVT.2733. Proc 3rd International Workshop on Insulin Resistance, New Orleans, LA, 2003, 2773:11–12 (Abstract)